

CLAIMS:

1. A method for in vitro maturation of immature human oocytes, comprising the steps of:

(a) inducing in a female human subject an increase in endogenous luteinizing hormone levels, said subject having not undergone an ovarian stimulation protocol prior to said inducing step;

(b) obtaining from said subject an immature oocyte; and

(c) culturing said oocyte until maturity.

2. The method according to claim 1, wherein step (a) comprises administering to said subject human chorionic gonadotrophin or luteinizing hormone, or both.

3. The method according to claim 1, wherein step (a) comprises administering to said subject human chorionic gonadotropin in an amount of about 5000 to about 20,000 IU.

4. The method according to claim 1, wherein said immature human oocyte comprises an M-I stage oocyte.

5. The method according to claim 1, wherein said immature human oocyte comprises a GV stage oocyte.

6. The method according to claim 1, wherein said immature human oocyte is cultured until it reaches M-II.

7. The method according to claim 1, wherein, in step (c), said immature human oocyte is essentially free of cumulus cells and is cultured in a culture medium comprising:

at least one inorganic salt;

essential amino acids or a source thereof;

an energy source; and

at least one growth factor.

8. The method according to claim 7, wherein said at
5 least one growth factor is selected from the group
consisting of fibroblast growth factor and epidermal growth
factor.

9. The method according to claim 7, wherein said
culture medium comprises both fibroblast growth factor and
10 epidermal growth factor.

10. The method according to claim 7, wherein said
culture medium further comprises at least one hormone.

11. The method according to claim 10, wherein said
hormone comprises insulin.

15 12. The method according to claim 11, wherein said
culture medium comprises from 0.5 mg/L to 50 mg/L insulin.

13. The method according to claim 7, wherein said
culture medium further comprises human transferrin.

14. The method according to claim 13, wherein said
20 culture medium comprises from 5 mg/L to 500 mg/L human
transferrin.

15. The method according to claim 7, wherein said
culture medium comprises from 0.0001 mg/L to 0.001 mg/L
fibroblast growth factor.

25 16. The method according to claim 7, wherein said
culture medium comprises from 0.0001 to 0.01 mg/L epidermal
growth factor.

17. The method according to claim 7, wherein said culture medium comprises one or more vitamins.

18. The method according to claim 17, wherein said one or more vitamins comprise biotin, D-Ca pantothenate,
5 choline chloride, folic acid, i-inositol, nicotinamide, pyroxidal·HCl, riboflavin, and thiamine·HCl.

19. The method according to claim 7, wherein said culture medium comprises hydrocortisone.

20. The method according to claim 7, wherein said
10 culture medium comprises selenite.

21. The method according to claim 7, wherein said inorganic salts comprise CaCl_2 , KCl, MgSO_4 , NaCl, NaHCO_3 , $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$.

22. The method according to claim 7, wherein said
15 energy source comprises D-glucose, or sodium pyruvate, or both D-glucose and sodium pyruvate.

23. The method according to claim 7, wherein said amino acids comprise alanine, arginine, asparagine, aspartic acid, cystine, glutamic acid, glycine, histidine,
20 isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

24. The method according to claim 7, wherein said culture medium comprises the inorganic salts, amino acids,
25 vitamins and other components as set forth in Table 1, and wherein each inorganic salt, amino acid, vitamin and other component is present in an amount of $\pm 50\%$ (weight/volume) of the amount specified in Table 1.

25. The method according to claim 24, wherein each inorganic salt, amino acid, vitamin and other component is present in said culture medium in an amount of $\pm 10\%$ (weight/volume) of the amount specified in Table 1.

5 26. The method according to claim 1, wherein, in step (c), said oocyte has a cumulus that is intact or at least partially intact, and/or said oocyte is cultured in the presence of cumulus cells.

10 27. The method according to claim 26, wherein said oocyte is cultured in a culture medium that is essentially free of epidermal growth factor.

28. The method according to claim 26, wherein said oocyte is cultured in a culture medium that is essentially free of fibroblast growth factor.

15 29. The method according to claim 26, wherein said oocyte is cultured in a culture medium that is essentially free of human transferrin.

30. The method according to claim 26, wherein said oocyte is cultured in a culture medium that is essentially
20 free of insulin.

31. The method according to claim 26, wherein said oocyte is cultured in a culture medium that is essentially free of selenite.

32. The method according to claim 26, wherein said
25 oocyte is cultured in a culture medium that is essentially free of hydrocortisone.

33. The method according to claim 26, wherein said oocyte is cultured in a culture medium that is essentially

free of epidermal growth factor, fibroblast growth factor, human transferrin, insulin, selenite, and hydrocortisone.

34. The method according to claim 26, wherein said oocyte is cultured in a culture medium comprising the
5 inorganic salts, amino acids, vitamins and other components as set forth in Table 2, and wherein each inorganic salt, amino acid, vitamin and other component is present in an amount of $\pm 50\%$ (weight/volume) of the amount specified in Table 2.

10 35. The method according to claim 34, wherein each inorganic salt, amino acid, vitamin and other component is present in said culture medium in an amount of $\pm 10\%$ (weight/volume) of the amount specified in Table 2.

36. The method according to claim 26, wherein said
15 oocyte is cultured in a culture medium consisting essentially of the inorganic salts, amino acids, vitamins and other components as set forth in Table 2.

37. The method according to claim 26, wherein said oocyte is cultured in a culture medium consisting of the
20 inorganic salts, amino acids, vitamins and other components as set forth in Table 2.

38. The method according to claim 1, wherein, prior to step (a), said subject has not been treated with a gonadotrophin releasing hormone agonist, human menopausal
25 gonadotrophin or follicle stimulating hormone.

39. A method for in vitro maturation of immature human oocytes, comprising culturing an immature human oocyte in a culture medium comprising:

at least one inorganic salt;

essential amino acids or a source thereof;

an energy source; and

at least one growth factor.

40. The method according to claim 39, wherein said at
5 least one growth factor is selected from the group
consisting of fibroblast growth factor and epidermal growth
factor.

41. The method according to claim 39, wherein said
culture medium comprises both fibroblast growth factor and
10 epidermal growth factor.

42. The method according to claim 39, wherein said
culture medium further comprises at least one hormone.

43. The method according to claim 42, wherein said
hormone comprises insulin.

15 44. The method according to claim 43, wherein said
culture medium comprises from 0.5 mg/L to 50 mg/L insulin.

45. The method according to claim 39, wherein said
culture medium further comprises human transferrin.

46. The method according to claim 45, wherein said
20 culture medium comprises from 5 mg/L to 500 mg/L human
transferrin.

47. The method according to claim 39, wherein said
culture medium comprises from 0.0001 mg/L to 0.001 mg/L
fibroblast growth factor.

25 48. The method according to claim 39, wherein said
culture medium comprises from 0.0001 to 0.01 mg/L epidermal
growth factor.

49. The method according to claim 39, wherein said culture medium comprises one or more vitamins.

50. The method according to claim 49, wherein said one or more vitamins comprise biotin, D-Ca pantothenate,
5 choline chloride, folic acid, i-inositol, nicotinamide, pyroxidal·HCl, riboflavin, and thiamine·HCl.

51. The method according to claim 39, wherein said culture medium comprises hydrocortisone.

52. The method according to claim 39, wherein said
10 culture medium comprises selenite.

53. The method according to claim 39, wherein said inorganic salts comprise CaCl_2 , KCl, MgSO_4 , NaCl, NaHCO_3 , $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$.

54. The method according to claim 39, wherein said
15 energy source comprises D-glucose, or sodium pyruvate, or both D-glucose and sodium pyruvate.

55. The method according to claim 39, wherein said amino acids comprise alanine, arginine, asparagine, aspartic acid, cystine, glutamic acid, glycine, histidine,
20 isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

56. The method according to claim 39, wherein said culture medium comprises the inorganic salts, amino acids,
25 vitamins and other components as set forth in Table 1, and wherein each inorganic salt, amino acid, vitamin and other component is present in an amount of $\pm 50\%$ (weight/volume) of the amount specified in Table 1.

57. The method according to claim 55, wherein each inorganic salt, amino acid, vitamin and other component is present in said culture medium in an amount of $\pm 10\%$ (weight/volume) of the amount specified in Table 1.

5 58. The method according to claim 39, wherein said culture medium consists essentially of the inorganic salts, amino acids, vitamins and other components as set forth in Table 1.

59. The method according to claim 39, wherein said
10 culture medium consists of the inorganic salts, amino acids, vitamins and other components as set forth in Table 1.

60. The method according to claim 57 or 58, wherein each inorganic salt, amino acid, vitamin and other component is present in an amount of $\pm 50\%$ (weight/volume) of the
15 amount specified in Table 1.

61. The method according to claim 57 or 58, wherein each inorganic salt, amino acid, vitamin and other component is present in an amount of $\pm 10\%$ (weight/volume) of the amount specified in Table 1.

20 62. The method according to claim 39, wherein said immature human oocyte is essentially free of cumulus cells.

63. A method for in vitro maturation of immature human oocytes, comprising culturing an immature human oocyte in a culture medium comprising the inorganic salts, amino acids,
25 vitamins and other components as set forth in Table 2.

64. The method according to claim 62, wherein said culture medium consists essentially of the inorganic salts, amino acids, vitamins and other components as set forth in Table 2.

65. The method according to claim 62, wherein said culture medium consists of the inorganic salts, amino acids, vitamins and other components as set forth in Table 2.

66. The method according to claim any one of claims
5 62-64, wherein each inorganic salt, amino acid, vitamin and other component is present in an amount of $\pm 50\%$ (weight/volume) of the amount specified in Table 2.

67. The method according to any one of claims 62-64,
10 wherein each inorganic salt, amino acid, vitamin and other component is present in an amount of $\pm 10\%$ (weight/volume) of the amount specified in Table 2.

68. The method according to claim 62, wherein said oocyte is cultured in the presence of cumulus cells